From the INTERNATIONAL BUREAU NOTIFICATION OF THE RECORDING BECKER, Konrad **OF A CHANGE Novartis AG** Patent- und Markenabteilung (PCT Rule 92bis.1 and Lichtstrasse 35 Administrative Instructions, Section 422) CH-4002 Basel SUISSE Date of mailing (day/month/year) 08 November 1999 (08.11.99) Applicant's or agent's file reference **IMPORTANT NOTIFICATION** PH/5 -30021/A International application No. International filing date (day/month/year) PCT/EP98/03279 02 June 1998 (02.06.98) 1. The following indications appeared on record concerning: X the applicant X the inventor the agent the common representative State of Nationality Name and Address State of Residence AU **BOUTSALIS**, Peter CH Lerchenstrasse 79 Telephone No. CH-4059 Basel Switzerland Facsimile No. Teleprinter No. 2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning: the person X the name the address the nationality the residence State of Nationality Name and Address State of Residence **BOUTSALIS**, Peter ΑU CH Känelmattstrasse 37 Telephone No. CH-4422 Arisdorf Switzerland Facsimile No. Teleprinter No. 3. Further observations, if necessary: 4. A copy of this notification has been sent to: the receiving Office the designated Offices concerned the International Searching Authority the elected Offices concerned

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland

the International Preliminary Examining Authority

Authorized officer

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.								
PH/5 -30021/A	ACTION								
International application No.	International filing date (day/month/year)		(Earliest) Priority Date (day/month/year)						
PCT/EP 98/03279	02/06/19	98	04/06/1997						
Applicant			· · · · · · · · · · · · · · · · · · ·						
NOVARTIS AG et al.	NOVARTIS AG et al.								
This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.									
This International Search Report consists of a total of 3 sheets. X It is also accompanied by a copy of each prior art document cited in this report.									
Certain claims were found unsearchable (see Box I).									
2. Unity of Invention is lacking (see Box II).									
3. The international application contains disclosure of a nucleotide and/or amino acid sequence listing and the international search was carried out on the basis of the sequence listing									
	filed with the international application.								
furnished by the applicant separately from the international application,									
but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.									
Tr.	anscribed by this Authority								
4. With regard to the title, the	e text is approved as submitte	d by the applicant							
X the	text has been established by this Authority to read as follows:								
PESTICIDE SCREENING S	SYSTEM								
5. With regard to the abstract,	- 1								
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PATENT COOPERATION THEAT

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Appli	cant's o	r age	nt's file reference			cation of Transmittal of Interr	
PH/5 -30021/A			\	FOR FURTHER ACTION	Preliminar	y Examination Report (Form	PCT/IPEA/416)
International application No.			cation No.	International filing date (day/month/year)		Priority date (day/month/)	year)
PCT/EP98/03279			279	02/06/1998		04/06/1997	
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3.	This re	port	contains indications rela	ating to the following items:			•
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	•		citations and explanati	ons suporting such statement	•		
VI 🗆 Certain documents cited							
VII							
	VIII		Certain observations of	n the international application			
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Form PCT/IPEA/409 (cover sheet) (January 1994)

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP98/03279

I.	Basis of the r port								
1.	This report has been drawn on the basis of (substitute sheets which have been furnished to the receiving Office response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.):								
	escription, pages:								
	15	as received o	n	10/03/1999	with letter of	09/03/1999			
	1-14,16-26	as originally t	iled						
	Claims, No.:								
	1-21	as received o	on	10/03/1999	with letter of	09/03/1999			
2.	The amendments hav	e resulted in th	e cancel	lation of:					
	☐ the description,	pages:							
	☐ the claims,	Nos.:							
	☐ the drawings,	sheets:							
3.	☐ This report has be considered to go	een establishe beyond the dis	d as if (so sclosure a	ome of) the amendmen as filed (Rule 70.2(c)):	nts had not been	made, since they have been			
4. Additional observations, if necessary:									
٧.	/. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement								
1.	Statement								
	Novelty (N)	Yes: No:	Claims Claims	1-21					
	Inventive step (IS)	Yes: No:	Claims Claims	1-21					

Industrial applicability (IA)

No: Yes:

No:

Claims 1-21

Claims

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP98/03279

2. Citations and explanations

see separate sheet

Section V:

Claims 1-21 refer to methods for testing asexually propagated plants within a plant screening program, wherein the propagation step is accomplished without passing through a callus phase or involving cell or protoplast culture.

In the light of the disclosure in the documents cited in the International Search Report, novelty and inventive step (Articles 33(2) and 33(3) PCT) of claims 1-21 is acknowledged.

What we claim is:

- 1. Test system for testing progeny plants, comprising
 - (a) asexually propagating progeny plant(s) from a mother plant without passing through a callus phase or involving cell or protoplast culture.;
 - (b) incorporating the so obtained progeny plant into a plant screening program; and
 - (c) monitoring the growth of the progeny plant.
- 2. Test system according to claim 1 wherein the propagation step is accomplished by
 - (a) cutting a short segment from a mother plant such that said segment is capable of directly regenerating into a whole and morphologically normal plant,
 - (b) transferring said excised segment to a suitable anchorage material; and
 - (c) regenerating said transferred segment into a whole and morphologically normal plan without passing through a callus phase or involving cell or protoplast culture.t
- 3. Test system according to claim 2, wherein said segment comprises a region that contains a high amount of actively dividing cells.
- 4. Test system according to claim 3, wherein said region comprises meristematic cells.
- 5. Test system according to claims 3 and 4, wherein said segment comprises a short root and shoot fragment.
- 6. Test system according to claim 2, wherein the anchorage material is
 - (a) an inert material such as vermiculite, perlite or plastic beads;
 - (b) a culture medium commonly applied in plant cultivation; or
 - (c) soil.

Reglaced by article 34

- Test system according to any of the previous claims wherein said test system is used 7. within a high through-put format.
- 8. Test system according to claim 1, comprising
 - (a) cutting a short segment from a mother plant such that said segment is capable of directly regenerating into a whole and morphologically normal plant,
 - (b)₁ dipping said segment into a know n concentration(s) of a pesticidecontaining solution; or, in the alternative
 - (b)₂ spraying said segment with a known concentration(s) of a pesticidecontaining solution;
 - (c) transferring the so treated plant explants to a suitable anchorage material;
 - (d) regenerating said explant into a whole and morphologically normal plant without passing through a callus/phase or involving cell or protoplast culture.; and
 - (e) monitoring the growth of the progeny plant.
- Method of rescuing plants showing an interesting trait or property after treatment with 9. a pesticide for further investigation comprising
 - (a) asexually propagating progeny plant(s) from a treated mother plant without passing through a callus phase or involving cell or protoplast culture.;
 - (b) incorporating the so obtained progeny plant into a plant screening program; and
 - (c) monitoring the growth of the progeny plant.
- Method according to claim 9, wherein the propagation step is accomplished by 10.
 - (a) cutting a short segment from a treated mother plant such that said segment is capable of directly regenerating into a whole and morphologically normal plant,
 - (b) transferring said excised segment to a suitable anchorage material; and
 - (c) regenerating said transferred segment into a whole and morphologically normal plant without passing through a callus phase or involving cell or protoplast culture..
- 11. Method according to claim 9 comprising

- (a) cutting a short segment from a treated mother plant such that said segment is capable of directly regenerating into a whole and morphologically normal plant,
- (b)₁ dipping said segment into a known concentration(s) of a pesticidecontaining solution; or, in the alternative
- (b)₂ spraying said segment with a known concentration(s) of a pesticidecontaining solution;
- (c) transferring the so treated plant explants to a suitable anchorage material;
- (d) regenerating said explant into a whole and morphologically normal plant without passing through a callus phase or involving cell or protoplast culture.; and
- (e) monitoring the growth of the progeny plant.
- 12. Method according to any of the previous claims, wherein the pesticide is selected from the group consisting of a herbicide, an insecticide and a fungicide.
- 13. Method for determining whether a resistance phenotype observed in a plant is due to a resistance trait or caused by other factors, comprising
 - (a) collecting the phenotypically resistant plant
 - (b) asexually propagating progeny plant(s) from said plant without passing through a callus phase or involving cell or protoplast culture.;
 - (c) incorporating the so obtained progeny plant into a plant screening program; and
 - (d) monitoring the growth of the progeny plant.
- 14. Method according to claim 13, comprising
 - (a) collecting the phenotypically resistant plant
 - (b) cutting a short segment from said plant such that said segment is capable of directly regenerating into a whole and morphologically normal plant,
 - (c)₁ dipping said segment into a known concentration(s) of a pesticidecontaining solution; or, in the alternative
 - (c)₂ spraying said segment with a known concentration(s) of a pesticidecontaining solution;
 - (d) transferring the so treated plant explants to a suitable anchorage material;

- (e) regenerating said explant into a whole and morphologically normal plant without passing through a callus phase or involving cell or protoplast culture.;
- (f) monitoring the growth of the progeny plant.
- 15. Method according to claims 13 and 14, wherein the resistance phenotype is observed after treating the plant with a pesticide.
- 16. Method according to claim 15, wherein the pesticide is selected from the group consisting of a herbicide, an insecticide and a fungicide.
- 17. Method according to any of the preceding claims, wherein the plant to be tested is a weed plant.
- 18. Method according to any of the preceding claims, wherein the plant to be tested is a crop plant.
- 19. Method according to any of the preceding claims, wherein the plant to be tested is a transgenic plant.
- 20. Use of a test system according to any one of claims 1-8 for rescuing plants showing an interesting trait or property after treatment with a pesticide for further investigation.
- 21. Use of a test system according to any one of claims 1-8 for determining whether a resistance phenotype observed in a plant is due to a resistance trait or caused by other factors.
- 22. Test-kit in a ready to use format comprising all devices necessary to carry out the test system according to any one of claims 1-8.
- 23. Test kit in a ready to use format according to claim 22 comprising
- (a) a cutting device, preferably a knife or sharp scissors to cut the weeds out of the ground, bags to put them in, pots, test tubes containing an anchorage material, preferably agar, soil, sand, vermiculite or a mixture of one or more of said components, etc, to grow them in, pesticide containing solution to spray with, a spraying device and an instruction manual;

- (b) a cutting device, preferably a knife or sharp scissors to cut the weeds out of the ground, bags to put them in, pots, test tubes containing an an anchorage material, preferably agar, soil, sand, vermiculite or a mixture of one or more of said components, etc, to grow them in, one or more container containing pesticide solution(s) with different concentrations of pesticide for dipping the plant cuttings into a known concentration(s) of pesticide and an instruction manual;
- (c) a cutting device, preferably a knife or sharp scissors to cut the weeds out of the ground, bags to put them in, pots, one or more container containing an appropriate anchorage material such as, for example, agar, soil, sand, vermiculite or a mixture of one or more of said components, wherein said anchorage material already contains uniformly distributed therein a pesticide solution with different concentrations of pesticide, and an instruction manual.
- 24. A test kit according to any of the previous claims, wherein the pesticide is selected from the group consisting of a herbicide, an insecticide and a fungicide.

phenotype can be rescued and further investigated thereby confirming that the individual plant or plants are truly resistant.

Structural genes which are preferably used to confer a particular resistance trait to a plant are those which code for proteins which are able to protect plants against pathogens (for example phytopathogenic fungi, bacteria, viruses etc.), herbicides (for example triazines, sulfonylureas, imidazolinones, triazolepyrimidines, bialaphos, glyophosates, etc.), fungicides, insecticides or disadvantageous environmental influences (for example heat, cold, wind, unfavorable soil conditions, moisture, dryness, etc.).

Within the scope of this invention, the use of transformed plants comprising structural genes associated with the control of plant pathogens and parasites are particularly preferred.

For example, resistance towards insects can be transferred by a gene which codes for a polypeptide which is toxic for insects and/or their larvae, for example the crystalline protein of *Bacillus thuringiensis*. Such genes are known and described, for example, in US-P 4,865,981, US-P 4,996,155, WO 89/07605, EP 213,318 and EP 186,379. A further class of vegetative insecticidal protein encoding DNA sequences is described in WO 94/21795 and WO 96/10083.

The protease inhibitors are a second class of proteins which mediate insect resistance. Protease inhibitors form a normal constituent of plant storage structures and for this reason are usually located in vacuoles or "protein bodies". Thus, it was possible to demonstrate that the Bowman-Birk protease inhibitor, which was isolated and purified from soya beans, inhibits the intestinal protease of Tenebrio larvae (Birk et al., 1963). The gene, which codes for a trypsin inhibitor from the common cowpea, is described in Hilder et al. (1987).

Within the scope of the present invention, plants comprising any *Bacillus thuringiensis* crystal protein, vegetative protein, protease inhibitor or any other insecticidal proteinencoding DNA sequences can be used within the testing system according to the invention, irrespective of their provenance (e.g. insecticidal proteins from non-plant sources or from purely synthetic sources).

In this connection, mention must also be made of hydrolytic enzymes which are either able on their own to bring about degradation of the cell walls of plant pathogens or else